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Characterization of 298 Patients with Lung Cancer Harboring *MET* Exon 14 Skipping Alterations

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ABSTRACT

Background: The hepatocyte growth factor receptor gene (*MET*) exon 14 skipping (*METex14*) has recently been described a potential driver alteration in lung cancer targetable by mesenchymal-to-epithelial transition factor (MET) tyrosine kinase inhibitors (TKIs).

Methods: Well-validated hybrid capture–based comprehensive genomic profiling was performed at the request of individual treating physicians.

Results: Of 11,205 lung cancers profiled by comprehensive genomic profiling, 298 (2.7%) carcinomas harbored alterations predicted to cause *METex14*, including adenosquamous (8.2%), sarcomatoid (7.7%), histologic subtype not otherwise specified (3.0%), adenocarcinoma (2.9%), squamous cell (2.1%), large cell (0.8%), and SCLC (0.2%). Acinar features were present in 24% of the *METex14* samples. Six cases (2%) harbored MET Y1003X mutations affecting binding of the MET-negative regulator, E3 ubiquitin protein ligase. The median age of all patients with *METex14* was 73 years (range 43–95) and 60% were female. Concurrent, murine double minute gene (*MDM2*) amplification, cyclin-dependent kinase 4 gene (*CDK4*) amplification, and *EGFR* amplification were observed in 35%, 21%, and 6.4% of patients with *METex14*, respectively. *KRAS* mutation was observed in 3% of cases. Concurrent *MET* amplification (*MET*amp) (median copy number 10) was identified in 15% of *METex14* samples. Significant differences in tumor mutational burden and type of the *METex14* alterations were observed between the *MET*amp and non-*MET*amp samples. Response to MET TKI was observed in both in patients with *MET*amp and in patients without *MET*amp *METex14*.

Conclusion: Diverse targetable *METex14* alterations were identified in patients with NSCLC across age groups, including elderly patients, and in all major NSCLC histologic subtypes with an overall frequency of 2.7%. These findings support the use of hybrid capture–based molecular profiling

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across NSCLC subtypes to identify patients who will potentially benefit from MET TKIs.

Keywords: *MET* exon 14 skipping; MET exon 14 alterations; Splice site mutations; Lung cancer; *MET* Y1003 mutation; Genomic profiling

Introduction

Tremendous advances have been achieved in the past decade in identifying druggable genomic drivers, including activating mutations of *EGFR* and *BRAF*, as well as fusions of the kinases anaplastic lymphoma receptor tyrosine kinase gene (*ALK*), *ROS1,* ret proto-oncogene (*RET*), and neurotrophic tyrosine kinase, receptor, type 1 gene (*NTRK*) in a significant fraction of patients with NSCLC.[1,2] The mesenchymal-to-epithelial transition factor (MET) receptor tyrosine kinase is a critical regulator of cell growth and development. Aberrant activation of MET in cancer can occur through several mechanisms, including hepatocyte growth factor receptor gene (*MET*) amplification (*MET*amp) or rearrangement, protein overexpression, overexpression of its ligand hepatocyte growth factor, and activating point mutations.[3,4] The multitargeted MET tyrosine kinase inhibitors (TKIs) crizotinib and cabozantinib have been approved for treatment of *ALK*-rearranged NSCLC and medullary and well-differentiated thyroid cancer, respectively.[5,6] Recently, two large-scale randomized phase 3 clinical trials with MET TKIs in unselected NSCLC have been unsuccessful.[7,8] However, these studies did not utilize *MET*amp or expression as entry criteria. Hence, a clear base of evidence for effective direct targeting of MET activation in solid malignancies remains elusive.

MET exon 14 alterations at RNA splice acceptor or donor sites have been recently described as a distinct mechanism of MET activation, leading to *MET* exon 14 skipping (*METex14*) and disruption of a DpYR motif, including Y1003, required for efficient binding of E3 ubiquitin protein ligase (CBL). These alterations have been shown to lead to increased MET stability and oncogenic potential, and to confer sensitivity to crizotinib.[9–14] Additionally, small series have demonstrated that patients with NSCLC harboring *METex14* alterations benefit from MET TKIs.[15,16] Recently we performed a large-scale analysis of *METex14* alterations in multiple cancers and found that these alterations are most common in lung adenocarcinomas (3%) and in other malignant lung neoplasms (2.3%).[17] Here we expand on that series to include more than 100 additional patients with lung cancer with *METex14* alterations and comprehensively characterize these cases by patient characteristics, tumor pathology, mutational burden, and coamplification of *MET*, murine double minute gene (*MDM2*), and *EGFR*. We additionally present eight previously unpublished responses to targeted therapy.

Methods

DNA was extracted from 40-μm formalin-fixed paraffin-embedded sections, and comprehensive genomic profiling (CGP) was performed on hybridization-captured, adaptor ligation–based libraries to a mean coverage depth of 820$\times$ for at least 3769 exons of 236 cancer-related genes plus 47 introns from 19 genes frequently rearranged in cancer. CGP for 11,205 consecutive lung cancers was ordered as part of routine clinical practice (August 2012–November 2015). Age, sex, stage, and histologic subtype were abstracted from the accompanying pathology report submitted by the treating physician. A subspecialty board–certified thoracic pathologist reviewed all *METex14*-positive cases. Testing was performed in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited reference laboratory (Foundation Medicine, Inc., Cambridge, MA). Patient samples were evaluated for genomic alterations (GAs), including base pair substitutions, insertions/deletions (indels), copy number alterations, and rearrangements, as described previously.[18] These GAs were then manually inspected to identify those likely to affect splicing of *MET* exon 14, or to delete the exon entirely as described previously.[17] Focal amplifications were called at segments with six or more copies (or $\geq$7 for triploid and $\geq$8 for tetraploid tumors) in samples with a purity greater than 20%.

Tumor mutational burden (TMB) was characterized as the number of somatic base substitution or indel alterations per megabase (MB) after filtering to remove known somatic and deleterious mutations. To calculate TMB, we used a novel algorithm that quantified the number of somatic mutations detected and extrapolated that value to the exome or genome as a whole (Frampton et al., Foundation Medicine Inc., unpublished data). Alterations with known (occurring as known somatic alterations in the Catalogue of Somatic Mutations in Cancer database) and likely (truncations in tumor suppressor genes) functional status were not counted. This filtering was performed to avoid upward skewing of mutational burden because CGP preferentially profiles genes known to be recurrently mutated in cancer. To calculate the mutation burden per MB, the total number of mutations was divided by the coding region target territory of the test, which is 1.11 MB for the current version. Ordinal

relationships were examined using the Mann-Whitney *U* test; categorical relationships were examined using Pearson's chi-squared test with Yates's continuity correction applied when applicable. Approval for this study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817).

Results

Clinical and Molecular Characteristics

Using CGP in the course of clinical care, we assayed tumor specimens from 11,205 patients with lung cancer (Supplementary Table 1). We identified *METex14* alterations in 298 patients (2.7%). The median age of patients with *METex14* was 73 years (range 47–95), with 79% age 65 years or older. The available clinical and molecular characteristics of all 298 patients are listed in Table 1. *METex14* alterations were most common in the adenosquamous histologic subtype (8.2%), followed by in the sarcomatoid histologic subtype (7.7%), not otherwise specified subtypes (3.0%), adenocarcinoma (2.9%), squamous cell carcinoma (2.1%), and large cell carcinoma (0.8%, two of 243) (Supplementary Fig. 1, Table 1). Through internal review of available hematoxylin and eosin–slides sarcomatoid features were identified in 17 additional *METex14* cases (Supplementary Fig. 2), and most (59%) of the not otherwise specified *METex14* samples favored adenocarcinoma. There was no notable difference in the types of *METex14* alterations among the various histologic subtypes. Patients with squamous and adenosquamous carcinoma had a slighter lower median age relative to the patients with *METex14* and other histologic subtypes ($p = 0.029$). Acinar features were identified in 23.8% of cases, followed by solid component (21.4%), micropapillary (9.7%), papillary (8.1%), and lepidic features (5.4%). Signet ring features were identified in four cases (1.3%), all of which were adenocarcinomas.

In the 298 cases with *METex14* alterations analyzed, 165 different variants predicted to affect *MET* exon 14 were detected. These included 157 base substitutions (24 unique) and 145 indels (139 unique), and they affected the splice acceptor site in 104 cases, the splice donor site in 191 cases, and the approximately 25–base pair (bp) intronic noncoding region immediately adjacent to the splice acceptor site in seven cases (Table 1 and Fig. 1). Five cases harbored multiple *METex14* alterations (Supplementary Table 2). In terms of the type of *METex14* alterations, 51.6% were base substitutions and 47.7% were indels. We also detected two cases with whole exon deletions of *MET* exon 14. Six cases of mutations affecting Y1003, which have been reported to impair CBL binding and MET degradation, and in one case leading to *METex14*, were identified.[19–21] All six of these patients were elderly females (Supplementary Table 3). In addition to the *METex14* alterations characterized in this series, we observed two cases with MET R1004G mutation. Similar to alteration of Y1003, mutations at D1002 and R1004 have been shown to disrupt CBL binding and MET degradation, and they may also be clinically relevant.[19]

Concurrent METamp

Concurrent *MET* amplification (*MET*amp) (mean copy number = 13 [range 6–59], median = 10) was detected in 44 cases (14.8%). This represents enrichment as compared with in the lung cancer cases in our data set that did not have *METex14* alterations, in which *MET*amp was observed in approximately 2.5% of cases. There was no significant difference in median age, sex, smoking history, or stage between the patients with *METex14* with or without concurrent *MET*amp (Table 2). However, the type of *METex14* alterations ($p = 0.017$) and TMB distribution ($p = 0.013$) were significantly different between patients with *METex14* with or without concurrent *MET*amp (Table 2). Non-*MET*-amp cases were most often characterized by a low mutational burden (zero to five mutations per MB), whereas *MET*amp cases were most often characterized by an intermediate low mutational burden (six to 10 mutations per MB) (Table 2). The median number of mutations per MB was 4.4 for non-*MET*amp and 6.8 for *MET*amp ($p = 0.007$).

Other Concurrent Driver Mutations

MDM2 amplification (*MDM2*amp) (mean copy number = 16 [range 6–100], median = 15) and cyclin-dependent kinase 4 gene (*CDK4*) amplification (*CDK4*amp) (mean copy number = 14 [range 6–54], median = 14), which have been previously shown to frequently co-occur with *METex14* alterations,[17] were also found in 103 cases (34.6%) and 63 cases (21.1%), respectively. There was no significant difference in most available characteristics between patients with and without *MDM2*amp (Supplementary Table 4). However, *CDK4*amp significantly correlated with *MDM2*amp in this series (Supplementary Table 4). Interestingly, we identified concurrent *EGFR* amplification (mean copy number = 11 [range 7–16], median =10) among 6.4% of *METex14* cases; erb-b2 receptor tyrosine kinase 2 gene (*ERBB2*) amplification and *KRAS* mutation were present in two (0.7%) and nine (3%) cases, respectively. There was one *METex14* case each with *EGFR* mutation (G719A), *BRAF* mutation (G466V), and concurrent echinoderm microtubule associated protein like 4 gene (*EML4*)-*ALK* fusion respectively.

TMB and Smoking Status

The average TMB in cases with *METex14* alterations was 6.9 mutations per MB (range zero to 197.9), which is

Table 1. Clinical and Molecular Characteristics of NSCLC Harboring *METex14* Alterations by Histologic Subtype

Patient Data	Values					
	All	AdenoCA	NOS	SqCC	AdenoSqCC	Sarcomatoid
Total Cases	11,205	7140	1659	1206	98	104
Cases with *METex14* alteration, n (%)[a]	298 (2.7)	205 (2.8)	49 (3.0)	25 (2.1)	8 (8.2)	8 (7.7)
Median age (range), y	72 (47-95)	73 (47-95)	75 (51-92)	69 (51-92)	67 (47-79)	75 (68-83)
Age, n (%)						
<age 65 y	61 (20)	46 (22)	6 (12)	7 (28)	2 (25)	0
≥age 65 y	235 (79)	157 (77)	43 (88)	18 (72)	6 (75)	8 (100)
Unknown	2 (0.7)	2 (1)	0	0	0	0
Sex, n (%)						
Male	118 (39.6)	79 (39)	19 (39)	10 (40)	4 (50)	4 (50)
Female	180 (60.4)	126 (61)	30 (61)	15 (60)	4 (50)	4 (50)
Smoking history, n (%)						
Yes	11 (5.4)	5 (2)	4 (8)	1 (4)	0	1 (12)
No	25 (8.4)	18 (9)	3 (6)	4 (16)	0	0
Unknown	262 (87.9)	182 (89)	42 (86)	20 (80)	8 (100)	7 (88)
Differentiation, n (%)						
Good	16 (9.9)	15 (7)	0	1 (4)	0	0
Moderate	38 (12.8)	32 (16)	2 (4)	1 (4)	3 (38)	0
Poor	107 (35.9)	58 (28)	27 (49)	13 (52)	3 (38)	4 (50)
Unknown	137 (46.0)	100 (49)	20 (41)	10 (40)	2 (25)	4 (50)
Histologic subtype, n (%)						
Adenocarcinoma	205 (68.8)	—	—	—	—	—
Adenosquamous	8 (2.7)	—	—	—	—	—
Squamous	25 (8.4)	—	—	—	—	—
Large cell	2 (0.7)	—	—	—	—	—
Sarcomatoid	8 (2.7)	—	—	—	—	—
SCLC	1 (0.3)	—	—	—	—	—
NSCLC (NOS)	49 (16.4)	—	—	—	—	—
Stage, n (%)						
I	12 (4.0)	10 (5)	1 (2)	0	1 (12)	0
II	27 (9.1)	19 (9)	2 (4)	4 (16)	1 (12)	0
III	15 (5.0)	7 (3)	4 (8)	2 (8)	0	2 (25)
IV	175 (58.7)	118 (58)	34 (69)	15 (60)	4 (50)	3 (38)
Unknown	69 (23.2)	51 (25)	8 (16)	4 (16)	2 (25)	3 (38)
METex14 alterations, n (%)[b]						
Base substitution splice donor	149 (49.1)	103 (50)	24 (49)	15 (60)	3 (38)	3 (38)
Indel splice acceptor	100 (32.9)	71 (35)	18 (37)	5 (20)	4 (50)	1 (12)
Indel splice donor	42 (13.8)	28 (14)	4 (8)	4 (16)	1 (12)	4 (50)
Base substitution splice acceptor	4 (1.3)	3 (1)	0	1 (4)	0	0
Base substitution noncoding adjacent splice acceptor	4 (1.3)	2 (1)	2 (4)	0	0	0
Indel noncoding adjacent splice acceptor	3 (1.0)	2 (1)	1 (2)	0	0	0
Whole exon 14 deletion	2 (0.7)	1 (0.5)	1 (2)	0	0	0
Concurrent *MET* amplification, n (%)						
Yes	44 (14.8)	32 (16)	9 (23)	2 (8)	0	1 (13)
No	254 (85.2)	173 (84)	40 (67)	23 (92)	8 (100)	7 (87)
Concurrent *MDM2* amplification, n (%)						
Yes	103 (34.6)	79 (39)	13 (27)	6 (36)	5 (63)	0
No	195 (65.4)	126 (61)	36 (73)	19 (64)	3 (37)	8 (100)
Concurrent *CDK4* amplification, n (%)						
Yes	63 (21.1)	50 (24)	9 (23)	2 (8)	1 (13)	0
No	235 (78.9)	155 (76)	40 (67)	23 (92)	7 (87)	8 (100)
Concurrent *EGFR* amplification, n (%)						
Yes	19 (6.4)	13 (6)	4 (8)	0	0	2 (25)
No	279 (93.6)	192 (94)	45 (92)	25 (100)	8 (100)	6 (75)

(continued)

Table 1. Continued

Patient Data	Values					
	All	AdenoCA	NOS	SqCC	AdenoSqCC	Sarcomatoid
Total Cases	11,205	7140	1659	1206	98	104
Mutational load (mutations per MB), n (%)						
Low (0-5)	168 (56.4)	117 (57)	27 (55)	13 (52)	5 (63)	4 (50)
Intermediate low (6-10)	95 (31.9)	63 (31)	16 (33)	9 (36)	3 (38)	3 (38)
Intermediate high (11-20)	31 (10.4)	21 (10)	6 (12)	3 (12)	0	1 (13)
High (>20)	4 (1.3)	4 (2)	0	0	0	0

[a]Percentage calculated from number in previous row.
[b]Includes four cases with two different *METex14* alterations and one case with three different *METex14* alterations (see Supplementary Table 2).
METex14, hepatocyte growth factor receptor gene exon 14 skipping; AdenoCA, adenocarcinoma; NOS, not otherwise specified; SqCC, squamous cell carcinoma; AdenoSqCC, adenosquamous carcinoma; Indel: insertion/deletion; *MDM2*, murine double minute 2 gene; *CDK4*, cyclin-dependent kinase 4 gene; MB, megabase.

slightly lower than the overall average of 10.7 mutations per MB for all lung cancers in our database. More importantly, most (56.4%) of the *METex14* tumor samples had low TMB (zero to five mutations per MB), and this was true regardless of histologic subtype (Table 1). Additionally, we assessed the relationship between smoking status and TMB. Smoking history was available for 36 of 298 patients. Among the 25 never-smokers, the mean TMB was 4.5 mutations per MB (range zero to 13.2, median = 3.3), whereas the mean TMB for the 11 patients with a history of smoking was 25.4 mutations per MB (range 0.8–197.7, median = 10.4).

Response of Patients with METex14 *NSCLC to MET TKIs*

Treatment outcomes were available for eight previously unpublished *METex14* cases treated with crizotinib. All eight patients in these cases experienced disease

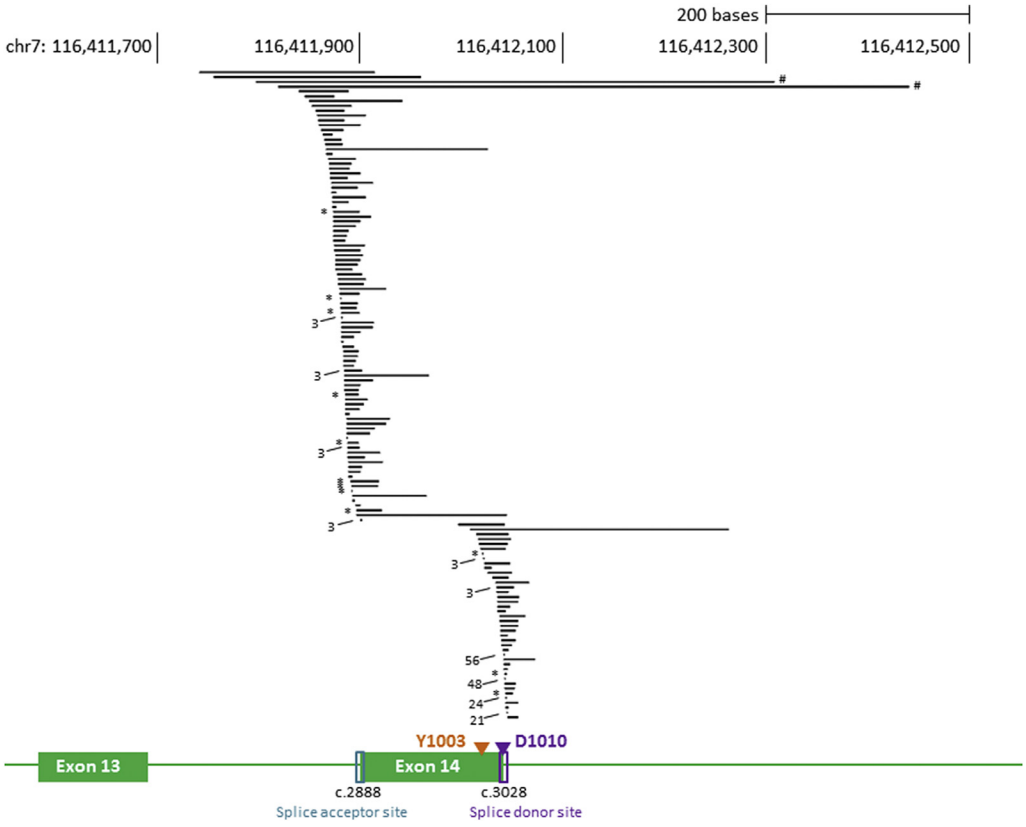

Figure 1. Schematic of all the genomic positions of hepatocyte growth factor receptor gene (*MET*) exon 14 skipping alterations. Alterations included base substitutions and insertions/deletions at the splice donor and acceptor sites, whole exon 14 deletion, and Y1003 mutation. Genomic positions with alterations occurring in more than one case are indicated with an asterisk (*) for two and the number of cases is greater than two. An octothorp (#) indicates samples with whole exon deletion of exon 14.

Table 2. Comparison of Clinical and Molecular Characteristics of Patients with *METex14* NSCLC with or without Concurrent *MET* Amplification

Patient Data	Without *MET* Amplification	With *MET* Amplification	*p* Value
No. patients	254	44	—
Median age (range), y	73 (47-95)	72 (49-87)	0.475
Sex, n (%)			0.353
Male	103 (41)	14 (32)	
Female	151 (59)	30 (68)	
Smoking history, n (%)			0.106
Yes	7 (3)	4 (9)	
No	23 (9)	2 (5)	
Unknown	224 (88)	38 (85)	
Histologic subtype, n (%)			<0.001
Adenocarcinoma	173 (68)	32 (73)	
Adenosquamous	8 (3)	0	
Squamous	23 (9)	2 (5)	
Large cell	2 (1)	0	
Sarcomatoid	7 (3)	1 (2)	
SCLC	1 (0.4)	0	
NSCLC (NOS)	40 (16)	9 (20)	
Differentiation, n (%)			0.046
Good	16 (6)	0	
Moderate	33 (14)	3 (7)	
Poor	87 (33)	22 (50)	
Unknown	118 (46)	19 (43)	
Stage at diagnosis, n (%)			0.300
I	12 (5)	0	
II	21 (9)	5 (11)	
III	14 (6)	1 (2)	
IV	145 (57)	30 (68)	
Unknown	62 (24)	8 (18)	
METex14 alterations, n (%)[a]			0.017
Base substitution splice donor	134 (53)	14 (32)	
Indel splice acceptor	84 (33)	16 (36)	
Indel splice donor	32 (13)	11 (25)	
Indel noncoding adjacent splice acceptor	1 (0.4)	2 (5)	
Base substitution splice acceptor	3 (1)	1 (2)	
Base substitution noncoding adjacent splice acceptor	4 (1)	0	
Whole exon 14 deletion	2 (0.8)	0	
Concurrent *MDM2* amplification, n (%)			0.203
Yes	92 (36)	11 (25)	
No	162 (64)	33 (75)	
Concurrent *CDK4* amplification, n (%)			0.128
Yes	58 (23)	5 (11)	
No	196 (77)	39 (89)	
Concurrent *EGFR* amplification, n (%)			0.643
Yes	15 (6)	4 (9)	
No	239 (94)	40 (91)	
Mutational burden (mutations per MB), n (%)			0.013
Low (0-5)	151 (59)	15 (34)	
Intermediate low (6-10)	76 (30)	19 (43)	
Intermediate high (11-20)	24 (9)	9 (20)	
High (>20)	3 (1)	1 (2)	

[a]Includes four cases with two different *METex14* alterations and one case with three different *METex14* alterations (see Supplementary Table 2).
METex14, hepatocyte growth factor receptor gene exon 14 skipping; y, year; NOS, not otherwise specified; Indel: insertion/deletion; *MDM2*, murine double minute 2 gene; *CDK4*, cyclin-dependent kinase 4 gene; MB, megabase.

control, including four partial responses and two complete responses (five responses are ongoing), and two cases with stable disease (Table 3 and Fig. 2). Responses to MET TKIs were seen in patients with *METex14* and concurrent *MET*amp, *MDM2*amp, or *CDK4*amp. One patient, a white never-smoking woman who presented with unresectable stage IIIB *METex14*-positive lung adenocarcinoma, received neoadjuvant crizotinib for 2 months with an

Table 3. Response of Patients with NSCLC with *METex14* to a MET TKI (Crizotinib)

Patient Case	Histologic Subtype	*METex14* Alteration	*MET* Amp	*MDM2* Amp	*CDK4* Amp	Biopsy Timing	Response to Crizotinib[a]
1	AdenoCA	3028+1_3028+1delG	Yes	No	Yes	After crizotinib	PR, 24 mo
2	AdenoCA	D1010Y	No	No	No	Before crizotinib	PR, 7 mo, ongoing
3	AdenoCA	3028+1delG	Yes	Yes	No	Before crizotinib	CR, 7 mo, ongoing
4	AdenoCA	D1010H	No	No	No	Before crizotinib	Stable disease, 4 mo, ongoing
5	AdenoCA	2888-16_2888-3del14	Yes	Yes	No	Before crizotinib	PR, 10 mo, ongoing
6	SqCC	2888-11_2904del28	No	No	No	Before crizotinib	PR
7	AdenoCA	2888-16_2888-13delTTCT	No	No	No	Before crizotinib	CR, 3 mo, ongoing
8	AdenoCA	3028 + 1G>A	No	No	No	Before crizotinib	Unresectable to resectable and NED after resection

[a]Investigator reported.
METex14, hepatocyte growth factor receptor gene exon 14 skipping; Amp, amplification; *MDM2*, murine double minute 2 gene; *CDK4*, cyclin-dependent kinase 4 gene; AdenoCA, adenocarcinoma; SqCC, squamous cell carcinoma; PR, partial response; mo, month; CR, complete response; NED, no evidence of disease.

excellent symptomatic and radiographic response that allowed her to undergo a complete tumor resection and mediastinal lymph node dissection, which revealed only extensive fibrosis and no viable cancer (Fig. 3).

Discussion

In this study, we identified *METex14* alterations in 298 cases (2.7%) among a large set of 11,205 lung cancers, indicating that as a potentially actionable driver *METex14* alteration is relatively frequent. Consistent with two retrospective studies, *METex14* alterations were enriched in a subset of lung sarcomatoid carcinomas included in this series (eight of 104 [7.7%]), although the frequency of *METex14* alterations in sarcomatoid lung cancer in our series is less than the 22% to 32% reported in recent smaller series.[22,23] However, our examination of the tissue was limited by the availability of only one hematoxylin and eosin–stained slide. *METex14* was also identified in a high frequency in adenosquamous carcinomas (eight of 98 [8.2%]). These data highlight the importance of CGP to identify previously underappreciated potentially druggable GAs in these histologic subsets of NSCLC. Importantly, *METex14* was identified in squamous cell carcinoma with a frequency of 2.1%, which has not been previously reported. There did not seem to be any notable difference in the available clinical and molecular characteristics among the major lung cancer histologic subtypes that harbor *METex14* alterations. Clinical responses to MET TKIs have now been reported across all NSCLC histologic subtypes, including large cell carcinoma.[15] Of note, most of the patients were elderly, with more than two-thirds of them age 65 years or older, which is consistent with a recent smaller study.[24] Additionally, in this study *METex14* alterations were identified in patients older than 90 years old, including in two of six patients with the ubiquitination-deficient Y1003 mutation. Hence, screening for *METex14* alterations in elderly patients may potentially allow a subset to benefit from anti–MET-targeted therapy with fewer side effects than chemotherapy. Advanced age and the nonadenocarcinoma histologic subtype should not be exclusion criteria for screening for *METex14* alterations.

The molecular alterations underpinning *METex14* events are diverse, involving base substitutions and indels at both the splice donor and acceptor sites of exon 14. In addition, we identified two cases harboring deletion of the whole exon 14 and 6 cases of Y1003X mutation. Y1003 is the binding site for the ubiquitin ligase CBL, which targets MET for degradation. Thus, Y1003 mutations are functionally analogous with the clinical sequelae of *METex14*,[25] although responses in patients with Y1003X mutations to MET-targeted therapies have not yet been reported. CGP allows for the identification of these diverse alterations, as well as any additional alterations in potential codrivers.

Among 36 patients whose smoking history was known, 25 (69%) were never-smokers, which is similar to the proportion observed with other major driver mutations in NSCLC.[26] Although smoking status was unknown in most of the cases in this report, patients with never-smoking status had significantly lower TMB (mean = 4.5 mutations per MB, median = 3.3 mutations per MB) compared with patients with a prior history of smoking (mean = 25.4 mutations per MB, median = 10.4

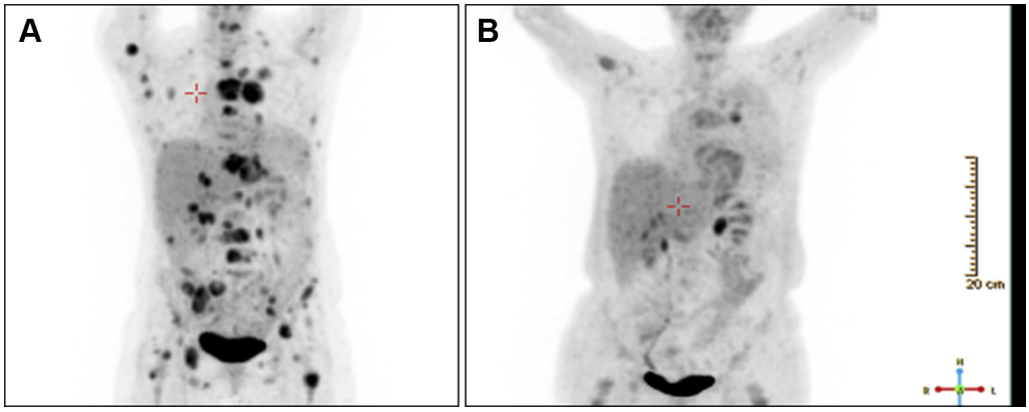

Figure 2. Complete response to crizotinib in patient (case 3) with hepatocyte growth factor receptor gene (*MET*) exon 14 skipping alteration. Fludeoxyglucose F 18 positron emission tomography scan images (*A*) before crizotinib treatment image and with obvious metastatic disease (*B*) after 6 weeks of crizotinib treatment, at which time fludeoxyglucose F 18 uptake has been diminished significantly.

mutations per MB) ($p = 0.046$). On the basis of the observation that most of the patients with *METex14* had a low (56%) or low intermediate (32%) mutation burden, we predict that most would be never-smokers.

Concurrent *MET*amp was identified in 15% of *METex14* cases. We did not observe a significant association between *MET*amp and stage IV disease ($p = 0.42$), as was recently reported on the basis of an analysis of 28 patients with *METex14* adenocarcinoma.[24] The caveat is that 23% of the patients in this series had an unknown stage; thus, this correlation between stage and *MET*amp will have to be investigated further.

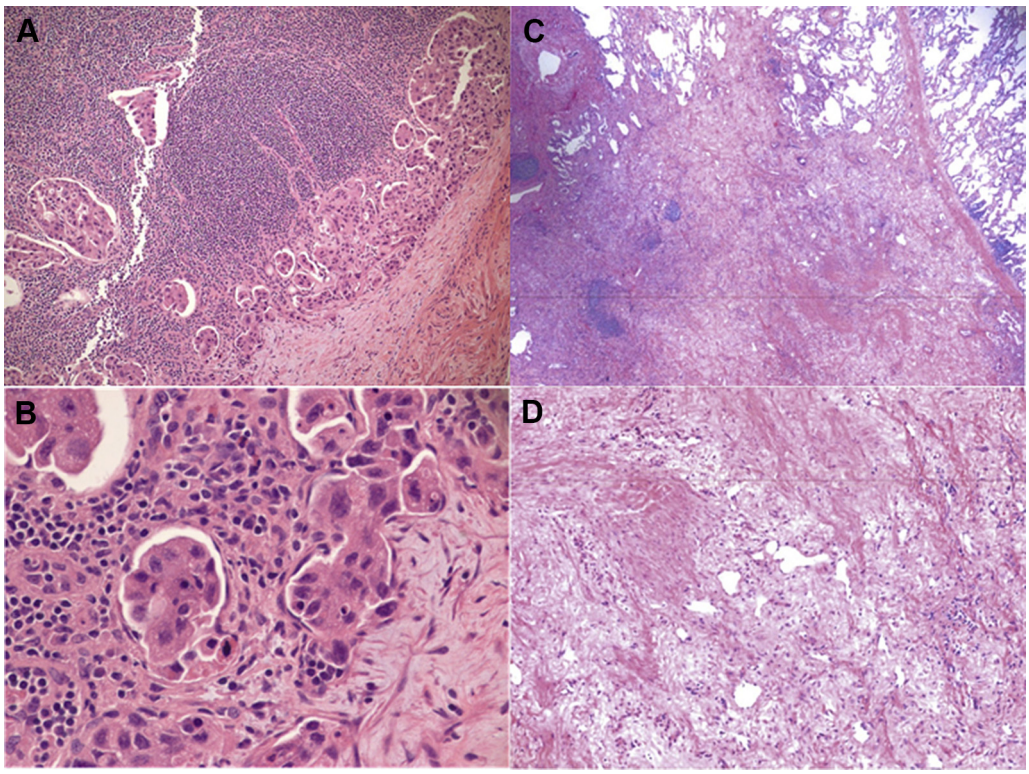

Figure 3. Pre-crizotinib treatment and post-crizotinib treatment hematoxylin and eosin stain from a mediastinal lymph node from an adenocarcinoma harboring hepatocyte growth factor receptor gene (*MET*) exon 14 skipping alteration. (*A* and *B*) Before crizotinib low-(40X) (*A*) and high-magnification (200X) (*B*) hematoxylin and eosin-stained images of a metastatic lesion in a mediastinal lymph node showing retraction artifact seen in tumors with micropapillary features. (*C* and *D*) After 2 months of neoadjuvant crizotinib low-(40X) (*C*) and high-magnification (100X) (*D*) images showing extensive fibrosis in the resected primary lung tumor.

Concurrent *MDM2*amp was the most common amplification identified in our series of *METex14* cases, and it has been identified as an oncogenic event in diverse tumor types acting through negative regulation of p53 function through ubiquination.[27,28] CBL is the ubiquitin ligase implicated as being primarily responsible for ubiquitination of MET due to the presence of a tyrosine kinase binding domain.[29] While METex14 protein lacks a tyrosine kinase binding domain, it is unknown whether *MDM2*amp is a compensatory mechanism to increase ubiquitination of MET through a different portion of MET protein or an event independent of *METex14* alterations. Patients with *METex14* both with and without *MET*amp or *MDM2*amp in this study and others have responded to MET TKIs.[15] Whether overall there is a differential response to MET TKIs according to *MET*amp or *MDM2*amp status in the context of *METex14* alterations needs to be investigated in a larger series of patients or clinical trials, especially given that there appears to be a significant difference in the TMB by *MET*amp status. Conversely, it will be important to investigate the frequency of *METex14* alterations among cases with de novo *MET*amp so as to truly identify the biology of *MET*amp and *METex14* alterations as separate driver mutations in NSCLC.

Lastly, we report eight previously unpublished *METex14* patients who responded to MET TKIs. For the first time, both pre–crizotinib treatment and post–crizotinib treatment tumor histologic information was available for one patient who demonstrated only extensive fibrosis after crizotinib treatment. MET is involved in the mesenchymal-to-epithelial transition, and the extensive fibrosis as opposed to apoptosis or necrosis may be related to the loss of this MET driver event. These data, together with previously published case reports/series, provide strong preliminary evidence that *MET*ex14 alterations are targetable driver mutations in lung cancer. Several phase 2 trials of MET TKIs targeting *METex14* alterations specifically are now ongoing, and the results from these prospective trials will be necessary to determine the response rate in this population. The high response rate to MET TKIs across patients with *METex14* alterations indicates that comprehensive genomic profiling in the course of clinical care is imperative to identify all possible molecular changes underpinning MET activation so that these patients can benefit from treatment with MET TKIs.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2016.06.004.

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